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Evaluation of Antidiarrhoeal activity of extract from roots of *Rumex hastatus* (Family: Polygonaceae) on experimental animals

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ABSTRACT

Rumex hastatus is fairly common small shrub, growing on dry slopes, rocks and walls between 700-2500 m, typically in North Indian hill station. Roots of *Rumex hastatus* have been used in Ayurveda and other folk medicines for the treatment of diarrhoea. To justify its folklore, present study was undertaken to investigate the antidiarrhoeal activity of the ethanolic extract from the roots of *Rumex hastatus* (EERH). Preliminary phytochemical screening, acute toxicity study and antidiarrhoeal activity of were studied on castor oil induced diarrhoea and normal gastrointestinal models of rats at 100, 150 and 200mg/kg body weight. The preliminary phytochemical screening of the ethanolic extract from the roots of *Rumex hastatus* (EERH) results with the presence of anthraquinone glycoside, tannins, flavonoids etc., LD₅₀ > 2000mg/kg. The doses of EERH significantly decreased (P<0.001) the total number of diarrhoeal faeces. PI decreases doses dependently (100, 150 and 200mg/kg orally) of EERH and was comparable with standard drug atropine sulphate. Conclusively, EERH has the antidiarrhoeal activity in experimental rats.

Key words: *Rumex hastatus*, Polygonaceae, Loperamide, Atropine.

INTRODUCTION

Diarrhoea is one of the main causes of high mortality rate in developing countries where over five million children under the age of five die annually from severe diarrhoeal diseases (WHO, 1996, Heinrich et al., 2005) and it is characterized by increased frequency of bowel movement, wet stool and abdominal pain (Maiti et al., 2007). Diarrhoeal disease is a leading cause of mortality and morbidity, especially in children in developing countries (Mani et al., 2010). A majority of diarrhoeal cases are due to bacterial enteropathogens, diarrhoeagenic *Escherichia coli* being the most common cause in developing countries. The two main bacterial groups causing traveler's diarrhoea are diarrhoeagenic *E. coli*, mainly enterotoxigenic and enteroaggregative (Adachi et al., 2001) and invasive bacterial pathogens like *Shigella*, *Campylobacter* and *Salmonella* (Hoge et al., 1998). Amongst the viral agents, rotavirus is the most common (Daswani et al., 2010). Although Oral Rehydration Therapy (ORT) has been very helpful in the treatment of diarrhoeal diseases, the oral rehydration solution (ORS) formulae supplemented with cooked rice powder was more effective than ORS treatment (Gore et al., 1992). Therefore, there is an urgent need for the intensification of research into medicinal plant claim to be effective in the management of diarrhoeal diseases (Mohammed et al., 2009). A vast majority of the people of developing countries relies on herbal drugs for the management of diarrhoea. Considering this fact the World Health Organization has constituted a diarrhoeal disease control programme, which

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includes studies of traditional medicinal practices, together with the elevation of health education and prevention approaches (Shaphiullah *et al.*, 2003).

Rumex hastatus is an annual, biennial and perennial herbs in the buckwheat family Polygonaceae. Members of this family are very common perennial herbs growing in acidic, sour soils mainly in the northern hemisphere. In India it is widely distributed in Himalayas, Jammu and Kashmir, Himachal Pradesh, Uttaranchal, and Kumaun. Roots of *Rumex hastatus* have grate mediation value are used in Ayurveda and other folk medicines for the treatment of different diseases and disorder such as rheumatoid arthritis, diarrhoea, dysentery, diuretic, wound healing, neoplasm, jaundice etc. (Pande *et al.*, 2007 and Zabta *et al.*, 2003). However, scientific evidence does not exist in literatures to corroborate the claims by traditional medicine practitioners of the therapeutic successes of the plant species.

The present study was undertaken to investigate the anti-diarrhoeal activity of the ethanolic extract from the roots of *Rumex hastatus* (EERH) to justify its folklore use in diarrhoea.

MATERIAL AND METHODS

Drug and chemicals

Atropine sulphate and loperamide (Sigma Chemical Co, St Louis, Mo, USA), castor oil (Qualikems Fine Chemicals Pvt. Ltd, New Delhi, India), normal saline (NaCl 0.9% w/v) and charcoal meal (10% activated charcoal in 100ml of 5% aqueous gum acacia) were used.

Plant Material

Roots of *Rumex hastatus* were collected in September 2010 from the Ranikhet, (U.P.), India and botanical identification was done by Dr. Tariq Husain, Scientist, National Botanical Research Institute (NBRI), Lucknow, India and voucher specimen has been preserved for further verification (ref no. 97817). The roots were separated, washed under running tap water, air dried under shade, coarsely powdered and kept in airtight container for further use.

Preparation of extracts

Dry powder was passed through the 40 mesh sieve. 300 gm of the powder was subjected to successive extraction in Soxhlet apparatus. The extract was evaporated under reduced pressure using rotary evaporator. Percentage yield of ethanolic extract from the roots of *R. hastatus* (EERH) was found to be 17.5 % w/w.

Preliminary phytochemical screening

The preliminary phytochemical screening of the EERH was carried out in order to ascertain the presence of its constituents (Evans, 2000).

Animals

Albino wistar rats (150-230g) of either sex were obtained from the animal house in IFTM university, school of Pharmacy, Moradabad. The animals were maintained in a room

having temperature 25°C with 12:12 hour light/dark cycle in polypropylene cages. The animals were fed with standard feed and water was given *ad libitum*. Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA (Ref No. CPCSEA/ac/2004).

Acute toxicity study

2000 mg/kg EERH was administered orally to three female rats. The general signs and symptoms of toxicity, intake of food and water and mortality were recorded for a period of two days and then for a period of 14 days (OECD, 2002).

Castor oil induced diarrhoea

Diarrhoea was induced in rats using castor oil. The group 1 (negative control) received in normal saline. Three test doses (100, 150 and 200 mg/kg body weight) of EERH were selected on a trial basis and administered orally by gavage to the animals of the group 2, group 3 and group 4. Group 5 (positive control) received loperamide at 3 mg/kg body weight as reference drug. Sixty minutes after drug treatment, each animal was administered 1 ml of castor oil orally. The number of defecation, the number of diarrhoeal feces and percentage of inhibition of diarrhoeal feces were calculated (Sunil *et al.*, 2001, Teke *et al.*, 2007 and Mani *et al.*, 2011).

Normal gastrointestinal transit

Normal gastrointestinal transit (motility) was investigated in rats as follows; Adult rats of both sexes were divided into five groups of six animals each to determine the effect of the EERH on normal intestinal transit of a marker meal. Group 1 received 10 ml/kg of distilled water, while animals in groups 2-4 were treated orally with the graded doses of EERH (100, 150 and 200 mg/kg respectively) and those in group 5 received 10 mg/kg of atropine sulphate. One hour after the administration of distilled water, extract or atropine sulphate, each animal was given 1 ml standard charcoal meal (10% activated charcoal suspension in 5% gum acacia). The rats were sacrificed 1 h after the administration of the charcoal meal, the abdomen was opened and the small intestine was immediately isolated. The length of the intestine from pylorus to the caecum (LSI) and the distance traveled by the charcoal (LM) were measured. The peristaltic index (PI) for each mouse was calculated, expressed as percentage of the distance traveled by the charcoal meal relative to the total length of the small intestine. The percentage inhibition relative to the control was also calculated as: $PI = LM/LSI \times 100\%$; Where, PI = Peristaltic Index; LM = Length of Charcoal Meal; LSI = Length of Small Intestine. % Inhibition = $[(Control - Test)/Control] \times 100$ (Aye-Than *et al.*, 1989, Odetola *et al.*, 2000 and Bakare *et al.*, 2011).

Statistical analysis

The results were expressed as mean \pm SEM and analyzed statistically to find out significance difference between control group against each test groups separately. The value of $P < 0.05$ was considered statistically significant.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical screening of the EERH results with the presence of anthraquinone glycoside, tannins, flavonoids etc.

Acute toxicity study

Oral administration of EERH produced no visible signs of toxicity in the animals at the 2000 mg/kg body weight of the rats. No mortalities were recorded. In addition, no toxic symptoms were observed and neither food nor water intake was found to be reduced during the period.

Castor oil induced diarrhea

The doses of EERH significantly decreased ($P < 0.001$) the total number of diarrhoeal faeces produced by administration of castor oil (5.2 ± 3.24 at the dose of 200 mg/kg) as compared to castor oil treated control group (10.4 ± 3.40) and comparable to the standard drug. The percentage of inhibition of castor oil induced diarrhoea in EERH treated rats was 50.00% at the dose of 200 mg/kg body weight of the rats and presented in table 1.

Table 1 Antidiarrhoeal activity of EERH against castor oil induced diarrhoea in rats.

Treatment	Dose (mg/kg, p.o.)	Total number of faeces	Number of diarrhoeal faeces	(%) inhibition of diarrhoea
Control	10ml/kg	14.6 ± 4.22	10.4 ± 3.40	-
EERH	100	12.4 ± 3.56	$9.2 \pm 2.96^*$	11.53
	150	$11.3 \pm 3.12^*$	$8.4 \pm 3.42^{**}$	19.23
	200	$8.4 \pm 2.94^{***}$	$5.2 \pm 3.24^{***}$	50.00
Loperamide	3	$8.2 \pm 2.42^{***}$	$4.8 \pm 2.86^{***}$	53.84

Data are expressed as mean \pm SEM (n = 6).

Where, * $p < 0.05$; ** $p < 0.01$ and *** $p < 0.001$ against control group.

Table 2 Effect of EERH on gastrointestinal motility of rats.

Treatment	Dose	Peristaltic Index (PI) (%)	Inhibition (%)
Control	10ml/kg	83.35 ± 3.21	-
EERH	100	$42.15 \pm 2.46^{**}$	49.43
	150	$30.25 \pm 2.98^{**}$	63.71
	200	$21.46 \pm 2.86^{***}$	74.25
Atropine Sulphate	10	$17.21 \pm 2.34^{***}$	79.35

Data are expressed as mean \pm SEM (n = 6). Where, ** $p < 0.01$ and *** $p < 0.001$ against control group.

Normal gastrointestinal transit

Peristalsis index decreases doses dependently (100, 150 and 200 mg/kg orally) of EERH and was comparable with standard drug atropine sulphate which was used as positive control and shown in table 2 (statistically significant ($P < 0.001$)). Therefore the produced percentage reduction was more at EERH 2000 mg/kg.

DISCUSSION

Ricinoleic acid is also reported to reduce active Na^+ and K^+ absorption and decrease Na^+ , K^+ ATPase activity in the small intestine and colon (Gaginella et al., 1975). As with other laxatives, castor oil changes the intestinal permeability and the histology (Mascolo et al., 1993).

The results of this study show that there has been a statistically significant reduction in the incident and severity of diarrhoea with the EERH in experimental animal models. EERH at 100, 150 and 200 mg/kg body weight doses significantly lowered several typical parameters of diarrhoea. EERH also showed significant anti-motility activity like the standard drug atropine sulphate. Further studies are required to confirm the underlying mechanism of the observed activity of the plant.

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